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MicroReview

# Rad3 and Sty1 function in *Schizosaccharomyces pombe*: an integrated response to DNA damage and environmental stress?

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## Summary

**In *Schizosaccharomyces pombe*, the Ataxia Telangiectasia-mutated (Atm)/Atm and Rad 3 Related (Atr) homologue Rad3 is an essential regulator of the response to DNA damage and stalled replication forks. Rad3 activates the downstream kinases Chk1 and Cds1. These kinases in turn inhibit cell cycle progression by mediating Cdc2 phosphorylation. Studies in both yeast and mammalian cells suggest additional roles for Rad3 in regulating cellular responses to environmental stress. In *S. pombe*, cellular responses to various environmental stresses are regulated primarily through the stress-activated MAP kinase p38 homologue Sty1. An important function of Sty1 is to drive cells rapidly through mitosis by facilitating the accumulation of Cdc25. Interestingly, Sty1 is activated simultaneously with Rad3 following exposure to UV radiation or ionizing radiation (IR). Similarly, exposure to environmental stresses induces the expression of *rad3*$^+$, *cds1*$^+$ and other checkpoint regulator genes. It is currently unclear how the pathways regulated by Sty1 and Rad3 and their opposing effects on mitosis are integrated. Recent studies suggest that Sty1 and Rad3 function together to regulate the expression of several stress response genes following exposure to IR. In this review, we discuss current knowledge on the interaction of Rad3/Atm and Sty1/p38 in regulating cellular responses to environmental stress and DNA damage.**

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## Rad3 in DNA damage checkpoint signalling

The checkpoint response was defined in the context of cell cycle arrest or delay following DNA damage or perturbed DNA replication (Hartwell and Weinert, 1989). To perform this task, sensor proteins directly or indirectly detect structural aberrations in the genome such as covalent DNA modifications or unreplicated DNA. Transmitter proteins (adapters and kinases) amplify the signal and transfer it to the effectors. The molecular sensing mechanisms that activate the DNA-dependent checkpoint pathway are not fully established. It has not been conclusively shown if the direct signal is constituted by the chemical modifications in damaged DNA itself, single-stranded regions, larger-scale structural changes in width or curvature of the damaged DNA region, chromatin modifications, or proteins bound to the site of DNA damage or stretch of unreplicated DNA. However, the ultimate source of the signal is chromosomal DNA.

A core set of proteins in the DNA-dependent checkpoint pathway is widely conserved among eukaryotes. The central signal transmitters belong to an unconventional protein kinase subfamily with sequence relatedness to phosphoinositol 3-kinases: Atm/Atr in human cells and their counterparts Tel1/Rad3 in fission yeast. DNA damage responses in mammalian cells are dependent on both Atm and Atr. However, one of the paralogues is mainly responsible for resistance to DNA-damaging agents in yeast (Rad3 in fission yeast; Mec1 in budding yeast), while the other paralogue (Tel1) has a minor role. The PCNA-like heterotrimeric '9-1-1' proteins (Rad9-Rad1-Hus1) are candidate sensor proteins. The MRN complex (Mre11–Rad50–Nbs1) binds to double-stranded breaks and has also been proposed to direct activation of the Atm protein (Lee and Paull, 2004). Upon activation of phosphatidylinositol 3-kinase-like kinases (PIKKs), some target proteins will be directly phosphorylated by them, whereas downstream conventional serine/threonine protein kinases will phosphorylate other targets of the pathway. In *Schizosaccharomyces pombe*, the

downstream checkpoint kinases are Chk1, with a role in G1 and G2, and Cds1 (homologous to human Chk2), which is active during S-phase (Walworth and Bernards, 1996; Lindsay *et al*., 1998). Adapter proteins promote the interaction between PIKK and downstream checkpoint kinases. Thus, *S. pombe* Crb2 (*Saccharomyces cerevisiae* Rad9, human 53BP1) gets recruited to double-stranded breaks and becomes phosphorylated; these events are required for efficient activation of Chk1 and DNA damage checkpoint signalling. In response to replication arrest, Mrc1 (yeast) or claspin (mammals), which are located at replication forks, are subject to PIKK-dependent phosphorylation. In fission yeast, this results in transmission of the checkpoint signal to Cds1 (Humphrey, 2000). For cell cycle regulation, the Cdk-activating protein phosphatase Cdc25 and the Cdk-inactivating protein kinases Wee1 and Mik1 have been shown to be effectors targeted by checkpoint signalling (Lundgren *et al*., 1991; Tourret and McKeon, 1996). It later became clear that in addition to its role in modulating cell cycle progression, checkpoint proteins are also required to initiate a transcriptional response (Allen *et al*., 1994; Gasch *et al*., 2001; Watson *et al*., 2004). Checkpoint signalling in *S. pombe* and other eukaryotes has been extensively reviewed (e.g. McGowan and Russell, 2004; Harrison and Haber, 2006).

## Rad3 in the environmental stress response

The role of Rad3 in regulating the cellular response to DNA damage has been well characterized (Humphrey, 2000). Recent studies suggest, however, that PIKKs play additional roles in regulating cellular responses to various environmental stresses. Interestingly, these studies also suggest that the response to DNA damage and its repair is fundamentally different in cells exposed to high levels of osmotic stress. The exposure of marine invertebrates and mammalian cells to hyperosmolarity (e.g. kidney cells) induces the accumulation of DNA double-stranded breaks (Kültz and Chakravarty, 2001; Dmitrieva *et al*., 2004; Dmitrieva *et al*., 2006). Similarly, exposure of log-phase *S. cerevisiae* cells to relatively high concentrations of sodium or potassium chloride induces base substitutions and frameshift mutations (Parker and von Borstel, 1987). Induction of DNA double-stranded breaks in yeast cells exposed to conditions of high osmolarity has not been demonstrated, however.

In mammalian cells, one of the Rad3 homologues, Atm, is activated by high concentrations of sodium chloride as well as by several other agents that do not cause DNA damage (Irarrazabal *et al*., 2004; Kurz and Lees-Miller, 2004). In the case of sodium chloride, Atm activation is likely to result at least in part from the accumulation of DNA breaks. Curiously, this activation of Atm does not result in induction of DNA repair. Instead, cells exposed to high sodium chloride concentrations adapt and continue to proliferate despite the presence of DNA double-stranded breaks (reviewed in Dmitrieva *et al*., 2004). High osmotic stress has been shown to induce nuclear export and cytoplasmic sequestration of Mre11 (D'Amours and Jackson, 2002; Dmitrieva *et al*., 2003). Once the cells are returned to isotonic medium, however, Mre11 relocates to the nucleus, Chk1 becomes phosphorylated and DNA repair is initiated (reviewed in Dmitrieva *et al*., 2005). Further studies have suggested that the Ku heterodimer, which is highly conserved in eukaryotes, is important for preserving the integrity of chromatin under conditions of high osmotic stress (Dmitrieva *et al*., 2005). It is unclear at present if osmotic stress directly damages DNA, or if double-stranded breaks accumulate under conditions of osmotic stress because DNA repair is inhibited, possibly because the breaks are somehow invisible to checkpoint and DNA repair proteins under hyperosmosis (Dmitrieva *et al*., 2005; Kültz, 2005).

The main function of Atm under conditions of high osmolarity appears to be the regulation of the stability, nuclear localization and activity of the TonEBP (tonicity response element binding protein)/OREBP transcription factor (Kültz and Burg, 1998; Kültz and Csonka, 1999). TonEBP is an important regulator of the adaptive mechanisms that are activated in mammalian cells under conditions of high osmolarity. Aside from Atm, several other protein kinases have also been shown to activate TonEBP (reviewed in Kültz, 2005). Interestingly, TonEBP activation by Atm occurs only in response to DNA damage that results specifically from osmotic stress (Irarrazabal *et al*., 2004; Zhang *et al*., 2005). An induction or accumulation of DNA double-stranded breaks in *S. pombe* cells exposed to osmotic stress has not been demonstrated, but a high degree of similarity exists between the cellular pathways of *S. pombe* and higher eukaryotes. It is thus likely that processing of DNA double-stranded breaks in *S. pombe* under osmotic stress conditions is similar to that observed in mammalian cells. At present, little is known about the mechanisms that facilitate cellular proliferation despite the presence of double-stranded breaks when cells are exposed to osmotic stress. We believe that *S. pombe* provides a simple but powerful model system to study the effects of osmotic stress on the cellular response to DNA damage.

Database searches indicate that *S. pombe* does not contain a TonEBP-like protein. In this yeast as in mammals, the expression of stress response genes is regulated to a large degree by stress-activated MAP kinases (SAPKs). Multiple mechanisms and stimuli can activate the p38 family MAP kinases. Both p38 and its *S. pombe* homologue Sty1 are activated in response to several environmental stresses including heat, oxidative

 

**Table 1.** Stress induction of genes regulated by Sty1 and Rad3.

| | Stress | | | | |
|---|---|---|---|---|---|
| Gene ID | Heat | Cadmium | $H_2O_2$ | Sorbitol | MMS |
| *gpx1*$^+$ | +++++ | +++ | ++++ | +++ | + |
| *meu8*$^+$ | – | – | +++++ | – | – |
| *obr1*$^+$ | – | – | +++ | – | + |
| *plr1*$^+$ | + | + | + | – | + |
| tms1$^+$ | ++++++++++ | – | ++++++++++ | – | – |

Stress response genes whose expression can be activated either by Sty1 or Rad3 in IR-exposed *S. pombe* cells (Watson *et al*., 2004). The relative fold induction of these genes in response to different environmental stresses is indicated (Chen *et al*., 2003).
MMS, methyl methane sulfonate.

stress, osmotic stress, heavy metals and UV radiation (Han *et al*., 1994; Millar *et al*., 1995; Degols *et al*., 1996; Chen *et al*., 2003). In addition, p38 is activated by a wide variety of biomolecules such as growth factors, hormones, inflammatory cytokines and lipopolysaccharides (Ono and Han, 2000). The best characterized signalling pathway upstream of Sty1 comprises the MAPK kinase (MAPKK) Wis1 and the MAPKK kinases Win1 and Wis4/Wak1. Upstream of those, a two-component system operates, including the membrane-bound histidine kinases Mak2 and Mak3, the phosphotransfer protein Mpr1, and the regulator Mcs4 (reviewed in Moye-Rowley, 2003). In keeping with the wide range of stimuli leading to p38 activation, there is a great number of upstream pathways funnelling into p38. Activation of target genes upon environmental stress is to a large extent controlled by the Sty1 pathway (Quinn *et al*., 2002; Chen *et al*., 2003; Weeks *et al*., 2006). Although activation of Rad3 by osmotic stress has not been reported, it is evident that Sty1 is activated following the exposure of *S. pombe* cells to both UV and ionizing radiation (IR) (Degols and Russell, 1997; Watson *et al*., 2004). An important question is if Rad3 cooperates with Sty1 to regulate activation of stress response genes in response to UV and/or IR in *S. pombe*. Recent studies suggest that this is indeed the case. Microarray studies (Watson *et al*., 2004) have demonstrated that in IR-exposed *S. pombe*, the increased expression of certain stress response genes (including *gpx1*$^+$, *meu8*$^+$, *obr1*$^+$, *plr1*$^+$ and *tms1*$^+$) was co-dependent on both Sty1 and Rad3 (Table 1), as induction of these genes was observed only in wild-type cells and *rad3*Δ or *sty1*Δ single mutants, but not in a *rad3*Δ *sty1*Δ double mutant. This observation suggests that a degree of functional redundancy exists between Rad3 and Sty1 in regulating the expression of these genes (Table 1). Similar studies on the role of Rad3 in the environmental stress response have not been performed, but expression of *gpx1*$^+$, *meu8*$^+$, *obr1*$^+$, *plr1*$^+$ and *tms1*$^+$ is increased in response to several environmental stresses other than IR (Chen *et al*., 2003) (Table 1). It will thus be interesting to determine if Sty1 and Rad3 cooperate to regulate the expression of these genes following exposure to environmental stresses. Surprisingly, the study by Watson *et al*. (2004) also suggested a role for Rad3 and Chk1 in the suppression of Sty1-induced genes. Following exposure to IR, *S. pombe rad3*Δ and *chk1*Δ mutants fail to suppress several Sty1-activated genes (e.g. *cgs1*$^+$, *rds1*$^+$, *srk1*$^+$/*mkp1*$^+$). It has not yet been determined if Rad3 and/or Chk1 similarly suppress the expression of these genes under environmental stress conditions.

Functional genomics studies in *S. pombe* have also demonstrated that genes encoding checkpoint regulators such as Rad3 and Cds1 are upregulated following exposure to various environmental stresses (Chen *et al*., 2003). The physiological function of this response is currently unclear. Induced overproduction of checkpoint regulators such as Cds1 causes cell cycle arrest (Murakami and Okayama, 1995; Martinho *et al*., 1998). Furthermore, both Rad3 and Cds1 regulate the activity and stability of proteins such as Mik1 and Wee1 that regulate Cdc2 phosphorylation and thus mitotic entry (Lindsay *et al*., 1998; Furnari *et al*., 1999; Christensen *et al*., 2000; Rhind and Russell, 2001). It remains to be determined, however, if these proteins play a role in modulating cell cycle progression under conditions of osmotic stress. More detailed studies on the importance of Rad3 and its substrates in regulating the stress response in *S. pombe* will establish an important and interesting area of research.

## Integrating Sty1 and Rad3 signalling in S. pombe following exposure to UV

Stress-activated MAP kinase pathways are activated by a wide range of stress conditions, some of which, such as UV light, IR or oxidative stress, are directly DNA-damaging. UV-induced activation of Jun N-terminal Kinase (JNK), a mammalian SAPK, will occur also in enucleated mammalian cells (Devary *et al*., 1993), demonstrating that the originating signal in this case cannot be DNA. Likewise, in

 

UV-exposed fission yeast cells, Sty1 activation occurs in response to the formation of free radicals rather than DNA damage itself. This was shown by co-treatment of UV-irradiated cells with N-acetyl cysteine (NAC), which is converted into the free radical scavenger glutathione; this abolished Sty1 activation. Furthermore, inactivation of DNA ligase, which causes accumulation of single-stranded breaks in DNA, did not result in Sty1 activation. Nonetheless, *sty1* mutants exhibit UV sensitivity similar to *rad24* mutants (Degols and Russell, 1997).

The simultaneous activation of both the Sty1-regulated stress response pathway, through mechanisms unrelated to DNA damage, and the checkpoint pathway, activated by DNA-dependent mechanisms, is thus crucial for maximal UV resistance. In mammalian cells, activation of the p38 pathway by UV irradiation, hyperosmosis, or inhibition of histone deacetylase activity, leads to inhibition of progression from late G2 or early prophase ('antephase') into mitosis, without activation of Atm (Bulavin *et al*., 2001; Matsusaka and Pines, 2004; Mikhailov *et al*., 2004). The two homologous pathways, however, exert distinct and opposing effects on cell cycle progression in fission yeast. The kinetics of hyperosmotic (0.6 M KCl) and UV (100–200 J $m^{-2}$) induced Sty1 activation are remarkably similar (Degols *et al*., 1996; Degols and Russell, 1997). Under both conditions, Sty1 activation is rapid and maximal at about 10 min post exposure. Activation of Sty1 results in an increased rate of mitosis, known as a 'mitotic burst', which is maximal at 2 h post exposure, leading to a shortened G2 phase and passage through mitosis at smaller cell size (Shiozaki and Russell, 1995; Kishimoto and Yamashita, 2000). Accumulation of cells in G1 is a common physiological response of *S. pombe* cells under other stress conditions such as stationary phase or nitrogen limitation, providing the opportunity to mate and form resilient ascospores. It is noteworthy that, although the p38-regulated MAP kinase-activated protein kinase 2 (MAPKAPK-2) is required for UV-induced phosphorylation of Cdc25B/C with ensuing cell cycle delays (Manke *et al*., 2005), this is not the case for the homologous Sty1-regulated kinase Srk1/Mkp1, which instead becomes activated in every cell cycle (Lopez-Aviles *et al*., 2005). Inhibition of MAPKAPK-2 induces sensitivity to UV (Manke *et al*., 2005), whereas *srk1* mutants are not UV-sensitive (Lopez-Aviles *et al*., 2005). It has not been directly shown whether Srk1 is activated through its UV- or hyperosmosis-induced phosphorylation by Sty1. The *sty1*$^+$ and *srk1*$^+$ genes have opposite direct effects on cell cycle progression and cell size: overexpression of *srk1*$^+$ confers cell elongation and inhibition of Cdc25, whereas *srk1* mutants divide at a reduced cell size and with a shortened G2 phase (Asp and Sunnerhagen, 2003; Lopez-Aviles *et al*., 2005). By contrast, *sty1* mutants are elongated with a G2 delay and reduced Cdc25 activity (Millar *et al*., 1995), while activation of Sty1 by UV or hyperosmosis as we have seen leads to advancement through mitosis. To rationalize these observations, there are two main options. The direct activation on cell cycle progression by Sty1 could overcome the inhibitory action of Srk1 either through exerting a stronger effect or by remaining active over a longer time window (Fig. 1). Formally, it could also be argued that Sty1-dependent phosphorylation of Srk1 under these particular conditions would be inhibitory, but we consider this less likely.

Activation of the Rad3-dependent DNA damage checkpoint is also rapid and phosphorylation of its downstream target Chk1 is detectable around 15 min after exposure to UV (Walworth and Bernards, 1996). Maximal activation of the G2 checkpoint, as measured by a substantial decrease in the number of mitotic or septated cells, occurs about 60 min after exposure to UV or IR (Jimenez *et al*., 1992; Rowley *et al*., 1992). As exposure to UV or IR results in the simultaneous activation of Sty1- and Rad3-regulated pathways, their respective effects on cell cycle progression are also likely to overlap.

Classical DNA damage checkpoint mutants such as *rad3*, *rad1*, *rad9*, *hus1*, *cds1* and *chk1* are generally believed to be unable to delay mitosis in response to DNA damage or stalled replication (Humphrey, 2000). This contention is not strictly accurate, however. An analysis of the literature reveals that these mutants not only fail to delay mitosis, but often actually increase their rate of entry into mitosis when exposed to DNA-damaging agents (Jimenez *et al*., 1992; Kanter-Smoler *et al*., 1995; Moser *et al*., 2000). In other words, classical checkpoint mutants often undergo a mitotic burst following exposure to IR, hydroxyurea (HU), bleomycin or UV. We hypothesize that at appropriate doses and in the absence of a functioning checkpoint, exposure to IR or UV induces a Sty1-dependent increase in mitotic rate. Indeed, *sty1*Δ *rad1*Δ double mutants do not display the mitotic burst observed in *rad1*Δ mutants following exposure to UV (J.P. Alao and P. Sunnerhagen, unpubl. data). These mutants are considerably more UV-sensitive than *rad1*Δ single mutants, again highlighting the importance of Sty1 in the response to this agent (J.P. Alao and P. Sunnerhagen, unpubl. data). The imposition of an artificial G2 arrest in IR-exposed *rad3* mutants provides only a very moderate protective effect (Jimenez *et al*., 1992). This is presumably because Rad3 enforces additional cellular responses such as DNA repair in addition to activating DNA damage checkpoints. Indeed, Rad3 is required for the expression of several genes involved in the regulation of S-phase and DNA repair (Watson *et al*., 2004). Earlier studies also demonstrated that Rad3 cooperates with Rad1, Rad9, Rad17 and Hus1 to induce the expression of the small subunit of ribonucleotide reductase when cells are exposed to DNA-damaging

 

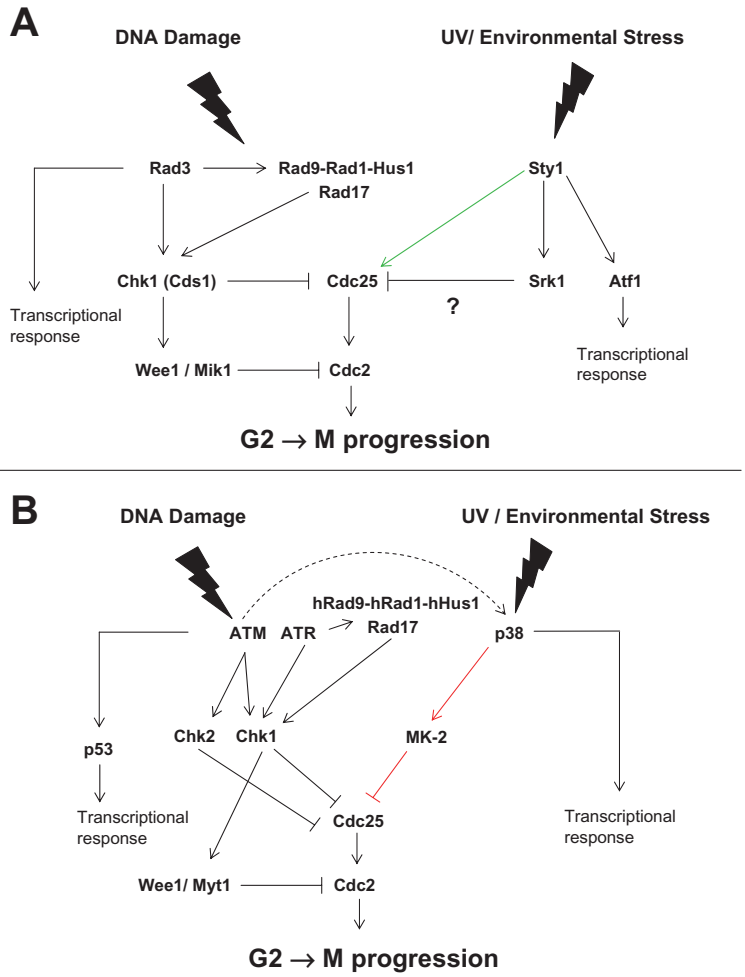


**Fig. 1.** Schematic representation of DNA damage checkpoint and cell cycle stress response pathways in *S. pombe* (A) and mammalian cells (B). It should be noted that in *S. pombe*, Sty1 activation accelerates mitosis, while p38 delays mitosis in mammalian cells. This checkpoint pathway has recently been shown to be important for the resistance of $p53^{-/-}$ cancer cells to DNA-damaging agents (Reinhardt *et al.*, 2007). The physiological role of Srk1/Mkp1-mediated Cdc25 inhibition in *S. pombe* remains uncertain.

agents (Harris *et al.*, 1996; Taylor *et al.*, 1996). By contrast, *S. pombe rad1*Δ mutants are DNA repair-competent and thus exhibit sensitivity to UV, mainly as a result of the absence of a fully functioning checkpoint (Rowley *et al.*, 1992). Rad1 is required to sustain the DNA damage checkpoint but not for its initiation, which is dependent on Rad3 (Martinho *et al.*, 1998; Nakamura *et al.*, 2005). Rad3 is thus able to regulate at least some cellular processes independently of Rad1. Consistent with this view, imposition of an artificial G2 arrest in UV-exposed *rad1*Δ mutants substantially enhances their survival (Al-Khodairy and Carr, 1992). Similarly, *chk1* mutants are defective only in the activation and maintenance of the G2 checkpoint and are considerably less sensitive to DNA-damaging agents than *rad3*, *rad1*, *rad9* and *hus1* mutants. It should be noted that in *chk1* mutants, Cds1 is able to activate both the S-phase checkpoint and recovery from stalled replication in a Rad3 and presumably Rad1-dependent manner (Boddy *et al.*, 1998). Interestingly, the sensitivity of *rad1* and *hus1* mutants can be suppressed even in the absence of alternative checkpoint activation (Dahlkvist *et al.*, 1995; Lieberman, 1995; Humphrey and Enoch, 1998; Kanoh *et al.*, 2003). Current models of the cellular response to DNA damage in *S. pombe* do not take into account the cell cycle effects of concomitant Sty1 activation, however. As noted above, DNA damage checkpoint mutants often increase their rate of mitosis in response to DNA damage in a manner that is dependent on Sty1, and so the suppressing effect may be through dampening of Sty1 rather than compensating for lacking Rad3 activity. It has not yet been demonstrated if prevention of mitotic burst activation is sufficient to substantially reduce the UV or IR sensitivity of *S. pombe* checkpoint mutants. Nonetheless, an increased rate of mitotic progression in the presence of damaged or unreplicated DNA undoubtedly enhances the lethality of DNA-damaging agents. In fact, overexpression of $sum1^{+}$ has previously been shown to abolish the Sty1-dependent cell cycle stress response in *S. pombe* (Humphrey and Enoch, 1998). Sum1 (also known as eIF3i, $eIF3^{p39}$) is a highly conserved WD-transducin repeat protein that probably plays a role in protein translation under environmental stress conditions (Humphrey and Enoch, 1998; Dunand-Sauthier *et al.*, 2002). Interestingly, $sum1^{+}$ overexpression suppresses the HU sensitivity of the

S-M checkpoint defective *cdc2-3w* mutant (Humphrey and Enoch, 1998). Based on these observations, the authors suggested that *sum1*$^+$ overexpression suppresses HU sensitivity by inactivating the cell cycle stress response. We propose that Sty1 activation in the absence of a functioning DNA damage checkpoint similarly enhances the lethality of other DNA-damaging agents in *S. pombe* by increasing the rate of mitotic progression.

Sty1 appears not to be required for proper checkpoint activation in response to UV-induced DNA damage (Degols and Russell, 1997). Its precise role in mediating cellular survival following UV exposure thus remains uncertain. Although it is clear that Sty1 is activated under certain conditions that damage DNA, it is not understood what alteration on the molecular level that is sensed. Thus, the lack of Sty1 activation in a DNA ligase mutant at the restrictive temperature suggests that Sty1 is not activated in response to unligated DNA (Degols and Russell, 1997). Generation of reactive oxygen species following UV exposure has thus been proposed as a likely source of the stimulus (Degols and Russell, 1997). This could also account for the activation of Sty1 following exposure to IR (Watson *et al*., 2004). Furthermore, high cellular levels of free radical scavengers abolish Sty1 activation following UV exposure (Degols and Russell, 1997), but free radical scavengers have not yet been shown to suppress the UV sensitivity of *sty1* mutants. The free radical hypothesis does not explain the activation of Sty1 by DNA alkylating agents or why the overexpression of *sum1*$^+$ suppresses the HU sensitivity of *cdc2-3w* mutants (Degols and Russell, 1997; Humphrey and Enoch, 1998). Indeed, HU has never been shown to activate Sty1. One possibility is that certain forms of DNA damage themselves generate reactive molecular species. It will therefore be interesting to see if Sty1 is activated in *cdc2-3w* mutants exposed to HU, and if free radical scavengers such as NAC can suppress the sensitivity of *cdc2-3w* mutants to this agent.

The precise function of Sty1 in regulating the cellular response of *S. pombe* to UV, IR and other DNA-damaging agents remains unclear, as well as the role of the various downstream proteins that are activated and/or induced. The Atf1 transcription factor is a major target of Sty1, but *atf1* mutants are not sensitive to UV (Degols and Russell, 1997). It has been suggested that Atf1 acts as a repressor of the *ctt1*$^+$ gene in *sty1* mutants as co-deletion of *sty1*$^+$ and *atf1*$^+$ restores *ctt1*$^+$ expression. Furthermore, *sty1 atf1* double mutants are less sensitive to UV than *sty1* single mutants. It should also be noted that Rad3 may function redundantly with Sty1 to regulate the expression of certain stress response genes (Watson *et al*., 2004). Sty1 also induces the expression and activation of Srk1, and overexpression of this protein has been shown to delay onset of mitosis (Smith *et al*., 2002; Lopez-Aviles *et al*., 2005). The deregulated expression of *srk1*$^+$ in *rad3* and *chk1* mutants exposed to IR (Watson *et al*., 2004) suggests that Srk1 may induce an alternative checkpoint in these mutants. UV-induced activation of Srk1 or UV sensitivity of *srk1* mutants has, however, not been reported. It is also not known if co-deletion of *srk1*$^+$ enhances the UV and/or IR sensitivity of DNA damage checkpoint mutants such as *chk1*. It will first be necessary to determine, however, if Srk1 protein levels are actually elevated in DNA damage checkpoint mutants. Similar studies will also be necessary to determine which downstream targets of Sty1 are actually important for regulating the DNA damage response in *S. pombe* (Table 1).

One evident function of Sty1 is to enable the cell to cope with the free radicals that are formed following exposure to UV and IR (Degols and Russell, 1997 and references therein). Activation of Sty1 results in the accumulation of Cdc25 and is crucial for the induction of a mitotic burst in response to nitrogen starvation, alkali or osmotic stress (Shiozaki and Russell, 1995; Kishimoto and Yamashita, 2000). Mutants with absent or defective *sty1* alleles do not accumulate Cdc25 or undergo mitotic burst when exposed to environmental stress. Instead, mutant cells arrest in G2, become greatly elongated and rapidly lose viability (Shiozaki and Russell, 1995; Kishimoto and Yamashita, 2000). Similarly, *sty1* mutants are unable to resume cell division following exposure to UV and the induction of the DNA damage checkpoint in G2 (Degols and Russell, 1997). We have also observed that in the absence of the DNA damage checkpoint, *sty1* mutants effectively resume cell division following exposure to UV (J.P. Alao and P. Sunnerhagen, unpubl. data). Sty1 may thus be required for cell cycle re-entry following checkpoint activation, which is consistent with its effect on Cdc25 and cell cycle progression. Following exposure to UV and/or IR, Sty1 activation is required to deal with increased levels of free radicals and the eventual resumption of cell division. At the same time, Rad3-dependent checkpoint activation is required to prevent mitosis in the presence of damaged DNA, but also to counter the induction of an increased mitotic rate (mitotic burst) by Sty1. In the absence of a functional DNA damage checkpoint, unregulated Sty1 activity results in an increase in the rate of mitosis despite the presence of DNA damage, with catastrophic consequences. An important function of the DNA damage checkpoint may thus be to facilitate the integration of Sty1 signalling with DNA repair processes. This integration allows cells to utilize the cytoprotective properties of Sty1 while simultaneously preventing its undesirable and potentially catastrophic effects on mitosis (Fig. 1).

## Conclusions

The Sty1-regulated stress response and Rad3-regulated DNA damage checkpoint pathways have been exten-

sively characterized. It is becoming increasingly evident that the co-activation of these pathways is important for both the response to environmental stress and DNA damage. To probe further into this area, it will be important to answer certain questions: is Rad3 activated by hyperosmosis in fission yeast, as is mammalian Atm? If so, are accumulated double-stranded breaks the reason, or can PIKKs be activated by other stimuli than DNA damage? If there is no TonEBP equivalent in fission yeast, does another protein serve an analogous role in co-ordinating the transcriptional response to hyperosmosis? What is the role of Sty1 in regulating the cell cycle responses of *S. pombe* to UV and other DNA-damaging agents? Specific prediction testing is possible: if Sty1 activation by UV is entirely attributable to oxidation products, then quenching of oxidative damage by adding a radical scavenger should partially alleviate the UV sensitivity of checkpoint mutants.

Recent studies in mammalian cells suggest that the DNA damage response in cells under conditions of hyperosmotic stress is fundamentally different from the conventional DNA damage response. We believe that the proper characterization of alternative mechanisms capable of abolishing the need for a functional DNA damage response is important for understanding how genomic instability and resistance to anti-cancer therapeutics develops. The effect of osmotic stress on the detection and repair of DNA damage in *S. pombe* has not been reported. There can be little doubt, however, that this versatile yeast will provide a powerful model to further characterize the interaction between stress and DNA damage response pathways.

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